## **CLAIMS**

- 1. Method for producing hemin proteins comprising the following steps:
- i) introducing, into plant cells, one or more nucleic acid molecule(s) each of which comprises at least one sequence encoding a protein component of a hemin protein of animal origin capable of reversibly binding oxygen or a variant or a portion of this protein component, and optionally a sequence encoding a selection agent;
- ii) selecting the cells containing the nucleic acid encoding the protein component of the hemin protein;
- iii) optionally propagating the transformed cells, either in culture or by regenerating whole transgenic or chimeric plants;
- iv) recovering, and optionally purifying, a hemin protein comprising a complex of the protein or proteins encoded by the abovementioned nucleic acid with at least one iron-containing porphyrin nucleus, or a plurality of these complexes.
- 2. Method according to Claim 1, characterized in that the hemin protein is a single polypeptide chain, for example myoglobin.
- 3. Method according to Claim 1, characterized in that the hemin protein is a heterooligomer, the nucleic acid(s) comprising the sequences encoding each of the various protein units.
- 4. Method according to Claim 3, characterized in that the hemin protein is human hemoglobin, or a derivative thereof, the nucleic acid(s) comprising sequences encoding  $\alpha$  and  $\beta$  globin, or variants of  $\alpha$  or  $\beta$  globin, the variants differing from the natural sequence in one or more amino acid substitution(s), deletion(s) or insertion(s).
- 5. Method according to Claim 4, characterized in that the sequences encoding the various protein units,  $\alpha$  and  $\beta$  globin, are contained within the same nucleic acid molecule.
- 6. Method according to Claim 4, characterized in that the sequences encoding the various protein units,  $\alpha$  and  $\beta$  globin, are contained within separate nucleic acid molecules.

- 7. Method according to any one of Claims 1 to 6, characterized in that the introduction of the nucleic acid molecule(s) is carried out by transformation of the nuclear genome of the plant cell.
- 8. Method according to Claim 7, characterized in that the sequence encoding the protein component comprises one or more sequence(s) encoding chloroplast targeting signals, or mitochondrial targeting signals.
- 9. Method according to Claim 7, characterized in that the sequence encoding the protein component comprises one or more sequence(s) encoding an N-terminal signal peptide and optionally a signal responsible for retaining the protein in the endoplasmic reticulum, or a vacuolar targeting signal.
- 10. Method according to any one of Claims 1 to 6, characterized in that the introduction of the nucleic acid is performed by a transformation of the mitochondrial or chloroplast genome.
- 11. Method according to any one of Claims 1 to 9, characterized in that the nucleic acid comprises, in addition to the coding sequence(s), sequences for regulation of transcription which are recognized by plant cells.
- 12. Method according to any one of Claims 4 to 11, characterized in that the coding sequence(s) encode(s) a hybrid molecule composed of at least the active parts of  $\alpha$ -globin and  $\beta$ -globin.
- 13. Method according to any one of Claims 1 to 12, characterized in that it comprises, between the propagation step and the recovery step, a step for detecting hemin proteins and in particular hemin proteins whose porphyrin nucleus consists of iron-containing protoporphyrin IX.
- 14. Method according to Claim 13, characterized in that during the step for propagating plant cells, iron-containing protoporphyrin IX is added to the culture medium used for growing the cells.
- 15. Hemin protein having the capacity to reversibly bind oxygen, characterized in that it comprises at least one iron-containing porphyrin nucleus, of plant origin, and a protein component comprising at least one polypeptide chain, of animal origin.
- 16. Protein according to Claim 15, characterized in that the iron-containing porphyrin nucleus is iron-containing protoporphyrin IX, or a protoporphyrin differing

from protoporphyrin IX in the nature of the side chains carried by the  $\beta$  atoms of the pyrrole rings.

- 17. Protein according to Claim 16, characterized in that the protein component comprises at least one  $\alpha$  and/or  $\beta$ -globin polypeptide chain, or variants thereof comprising one or more amino acid substitution(s), deletion(s) or insertion(s), the hemin protein being capable of binding oxygen reversibly.
- 18. Protein according to Claim 17, characterized in that the  $\alpha$  or  $\beta$ -globin chain, or variants thereof, comprises in addition a chloroplast targeting signal, a mitochondrial targeting signal, or a N-terminal signal peptide optionally in combination with a signal responsible for retaining protein in the endoplasmic reticulum, or a vacuolar targeting signal.
- 19. Protein according to Claim 17, characterized in that each polypeptide chain lacks NH<sub>2</sub>-terminal methionine.
- 20. Protein according to any one of Claims 17 to 19, characterized in that the protein component comprises at least four polypeptide chains of  $\alpha$  and/or  $\beta$  globin or variants thereof, each polypeptide chain being bound to an iron-containing protoporphyrin nucleus.
- 21. Protein according to Claim 20, characterized in that it comprises 2  $\alpha$ -globin chains and 2  $\beta$ -globin chains, or variants thereof.
- 22. Protein according to any one of Claims 15 to 21, characterized in that it binds oxygen with an affinity of between 7 and 40 mm Hg, preferably 15 to 20 mm Hg.
- 23. Nucleic acid comprising:
- i) one or more sequence(s) encoding a protein component of an animal hemin protein, the said protein having the capacity to reversibly bind oxygen, and
- ii) sequences for regulation of transcription which are recognized by a plant cell, comprising a promoter and sequences for regulation of termination, and
- iii) one or more sequence(s) encoding a targeting signal of plant origin.
- 24. Nucleic acid according to Claim 23, characterized in that the regulatory sequences comprise one or more promoter(s) of plant origin.

- 25. Nucleic acid according to Claim 23 or 25, characterized in that the sequences encoding the targeting signal encode a mitochondrial or chloroplast targeting peptide called "transit" peptide.
- 26. Nucleic acid according to Claim 23 or 24, characterized in that the sequences encoding the targeting signal encode an N-terminal signal peptide of plant origin, optionally in combination with a sequence encoding an endoplasmic retention signal or a vacuolar targeting signal.
- 27. Nucleic acid according to any one of Claims 23 to 26, characterized in that the coding sequence encodes human  $\alpha$  or  $\beta$ -globin, or a variant thereof differing from the natural sequence in one or more amino acid substitution(s), deletion(s) or replacement(s), or a portion of human  $\alpha$  and/or  $\beta$ -globin.
- 28. Nucleic acid according to any one of Claims 23 to 27, comprising, in addition, one or more intron(s), preferably of plant origin.
- 29. Nucleic acid according to any one of Claims 23 to 28, characterized in that the sequence encoding the protein component is a cDNA.
- 30. Vector comprising one or more nucleic acid molecule(s) according to any one of Claims 23 to 29.
- 31. Plant cells transformed in a stable manner by the nucleic acid according to any one of Claims 23 to 29.
- 32. Plant cells capable of producing one or more hemin protein(s) according to any one of Claims 15 to 22.
- 33. Plant cells according to Claim 32, characterized in that they comprise nucleic acid comprising one or more sequence(s) encoding a protein component of the said hemin protein in association with one or more sequence(s) for regulation of transcription recognized by the cell.
- 34. Plant cells according to any one of Claims 31 to 33, characterized in that they are a culture of plant cells, for example in liquid medium or immobilized cells, or a root culture.
- 35. Plant cells according to any one of Claims 31 to 33, characterized in that they are cells which form part of a whole transformed plant.

- 36. Chimeric or transgenic plant capable of producing one or more hemin protein(s), for example hemoglobin or a derivative thereof, characterized in that it comprises cells according to any one of Claims 31 to 33.
- 37. Seeds of transgenic plant according to Claim 36.
- 38. Pharmaceutical product comprising one or more hemin protein(s) according to any one of Claims 15 to 22 in association with a physiologically acceptable excipient.
- 39. Hemin proteins according to any one of Claims 15 to 22 for use as medicament.
- 40. Hemin protein according to Claim 39, for use in the treatment of conditions requiring an improvement in the transport of oxygen in the blood.
- 41. Use of a hemin protein according to any one of Claims 15 to 22 for the preparation of a medicament for the treatment of conditions requiring an improvement in the transport of oxygen in the blood.
- 42. Use of a hemin protein according to any one of Claims 15 to 22 in an industrial, cosmetic product or as chemical reagent.